C54

71. (Amended) The method of claim 3 wherein the dominant negative allele comprises a truncation mutation at codon 134.

In the Specification

Please substitute the following paragraphs, headings, or tables for those in the specification:

At page 18, substitute the following paragraph:

C6

The use of over expressing foreign mismatch repair genes from human and yeast such as PMS1, MSH2, MLH1, MLH3, etc. have been previously demonstrated to produce a dominant negative mutator phenotype in bacterial hosts (35, 36, 37). In addition, the use of bacterial strains expressing prokaryotic dominant negative MMR genes as well as hosts that have genomic defects in endogenous MMR proteins have also been previously shown to result in a dominant negative mutator phenotype (29,32). However, the findings disclosed here teach the use of MMR genes, including the human PMSR2 and PMSR3 gene (ref 19), the related PMS134 truncated MMR gene (ref 32), the plant mismatch repair genes and those genes that are homologous to the 134 N-terminal amino acids of the PMS2 gene which include the MutL family of MMR proteins and including the PMSR and PMS2L homologs ~~desribed~~ described by Hori et.al. (accession number NM_005394 and NM_005395) and Nicolaides (reference 19) to create hypermutable microbes. In addition, this application teaches the use of DNA mutagens in combination with MMR defective microbial hosts to enhance the hypermutable production of genetic alterations. This accentuates MMR activity for generation of microorganisms with commercially relevant output traits such as but not limited to recombinant protein production strains, biotransformation, and bioremediation.

At pages 19-20, substitute the following paragraph:

C7

Bacterial expression constructs were prepared to determine if the human PMS2 related gene (hPMSR3) (19) and the human PMS134 gene (32) are capable of inactivating the bacterial MMR activity and thereby increase the overall frequency of genomic hypermutation, a consequence of which is the generation of variant sib cells with novel output traits following host selection. Moreover, the use of regulatable expression vectors will allow for suppression of dominant negative MMR alleles and restoration of the MMR pathway and genetic stability in hosts cells (43). For these studies, a plasmid encoding the hPMS134 cDNA was altered by polymerase chain reaction (PCR). The 5' oligonucleotide has the following structure: 5'-ACG CAT ATG GAG CGA GCT GAG AGC TCG AGT-3' (SEQ ID NO: 1) that includes the NdeI restriction site CAT ATG. The 3'-oligonucleotide has the following structure: 5'-GAA TTC TTA TCA CGT AGA ATC GAG ACC GAG GAG AGG GTT AGG GAT AGG CTT ACC AGT TCC AAC CTT CGC CGA TGC-3' (SEQ ID NO: 2) that includes an EcoRI site GAA TTC and the 14 amino acid epitope for the V5 antibody. The oligonucleotides were used for PCR under standard conditions that included 25 cycles of PCR (95^{0}C for 1 minute, 55^{0}C for 1 minute, 72^{0}C for 1.5 minutes for 25 cycles followed by 3 minutes at 72^{0}C). The PCR fragment was purified by gel electrophoresis and cloned into pTA2.1 (InVitrogen) by standard cloning methods (Sambrook et al., Molecular Cloning: A Laboratory Manual, Third Edition, 2001), creating the plasmid pTA2.1-hPMS134. pTA2.1-hPMS134 was digested with the restriction enzyme EcoRI to release the insert (there are two EcoRI restriction sites in the multiple cloning site of pTA2.1 that flank the insert) and the fragment filled in with Klenow fragment and dNTPs. Next, the fragment was gel

purified, then digested with NdeI and inserted in pT7-Ea that had been digested with NdeI and BamHI (filled with Klenow) and phosphatase treated. The new plasmid was designated pT7-Ea-hPMS134. The following strategy, similar to that described above to clone human PMS134, was used to construct an expression vector for the human related gene PMSR3. First, the hPMSR3 fragment was amplified by PCR to introduce two restriction sites, an NdeI restriction site at the 5'- end and an Eco RI site at the 3'-end of the fragment. The 5'-oligonucleotide that was used for PCR has the following structure: 5'-ACG CAT ATG TGT CCT TGG CGG CCT AGA-3' (SEQ ID NO: 3) that includes the NdeI restriction site CAT ATG. The 3'-oligonucleotide used for PCR has the following structure: 5'-GAA TTC TTA TTA CGT AGA ATC GAG ACC GAG GAG AGG GTT AGG GAT AGG CTT ACC CAT GTG TGA TGT TTC AGA GCT-3' (SEQ ID NO: 4) that includes an EcoRI site GAA TTC and the V5 epitope to allow for antibody detection. The plasmid that contained human PMSR3 in pBluescript SK (19) was used as the PCR target with the hPMS2-specific oligonucleotides above. Following 25 cycles of PCR (95^{0}C for 1 minute, 55^{0}C for 1 minute, 72^{0}C for 1.5 minutes for 25 cycles followed by 3 minutes at 72^{0}C). The PCR fragment was purified by gel electrophoresis and cloned into pTA2.1 (InVitrogen) by standard cloning methods (Sambrook et al., Molecular Cloning: A Laboratory Manual, Third Edition, 2001), creating the plasmid pTA2.1-hR3. pTA2.1-hR3 was next digested with the restriction enzyme EcoRI to release the insert (there are two EcoRI restriction sites in the multiple cloning site of pTA2.1 that flank the insert) and the fragment filled in with Klenow fragment and dNTPs. Then, the fragment was gel purified, then digested with NdeI and

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inserted in pT7-Ea that had been digested with NdeI and BamHI (filled with Klenow) and phosphatase treated. The new plasmid was designated pT7-Ea-hR3.

At page 25, substitute the following table:

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TMT 12/24/02

STRAIN	# CELLS SEEDED	AMP^R colonies	KAN^R colonies	FREQUENCY ~~CY~~
DH10B VEC	50,000	62,000	0	0
DH10B PMS134	50,000	43,146	23	5.3×10^{-4}
BL21 VEC	500,000	520,800	0	0
BL21 T7-Ea-PMS134V5	500,000	450,000	2,245	4.9×10^{-3}
BL21 T7-Ea-PMSR3V5	500,000	500,000	1,535	3.1×10^{-3}

At page 25-26, substitute the following paragraph:

C9

Using the same protocol as listed above and the same cloning strategy, a truncated PMS2 homolog from the *Arabidopsis thaliana* plant, which was cloned by degenerate PCR from an *Arabidopsis thaliana* cDNA library (Strategene), was found to give a similar enhancement of genetic hypermutability in DH5alpha bacteria Figure 5. For detection purposes, we fused a FLAG epitope to the C-terminus of the PMS134 polypeptide using PCR and an antisense primer directed to the 134 codon region of the *Arabidopsis* PMS2 homolog followed by a FLAG epitope and 2 termination codons. The resultant fusion was termed ATPMS134-flag. The AT PMS134-flag gene was then cloned into the IPTG-inducible TACLAC expression vector and transfected into DH5alpha cells. Western blot of bacteria transfected with an IPTG-inducible expression vector carrying a truncated version (codons 1-134) of the Arabidopsis thaliana PMS2 homolog using the anti-

FLAG antibody demonstrated the inducibility and steady-state protein levels of the chimeric gene. Figure 5A shows the western blot containing protein from an untransfected cell (lane 1) and a bacterial clone expressing the Arabidopsis PMS2-134 truncated protein (lane 2). Following the mutagenesis protocol described above, bacterial cells expressing the ATPMS134 protein were found to have an increase in the number of KAN resistant cells (12 clones) in contrast to cells expressing the empty vector that yielded no KAN resistant clone.

At page 37, substitute the following two headings:

C10 Yeast MLH1 cDNA (accession number U07187) (SEQ ID NO: 5)

C11 Yeast MLH1 protein (accession number U07187) (SEQ ID NO: 15)

At page 38, substitute the following two headings:

C12 Mouse PMS2 protein (SEQ ID NO: 16)

C13 Mouse PMS2 cDNA (SEQ ID NO: 6)

At page 39, substitute the following heading:

C14 human PMS2 protein (SEQ ID NO: 17)

At page 40, substitute the following headings

C15 Human PMS2 cDNA (SEQ ID NO: 7)

At page 41, substitute the following two headings:

C16 human PMS1 protein (SEQ ID NO: 18)

932

C17 Human PMS1 cDNA (SEQ ID NO: 8)

At page 42, substitute the following heading:

C18 human MSH2 protein (SEQ ID NO: 19)

At page 43, substitute the following heading:

C19 Human MSH2 cDNA (SEQ ID NO: 9)

At page 44, substitute the following two headings:

C20 human MLH1 protein (SEQ ID NO: 20)

C21 Human MLH1 cDNA (SEQ ID NO: 10)

At page 45, substitute the following three headings:

C22 hPMS2-134 protein (SEQ ID NO: 21)

C23 hPMS2-134 cDNA (SEQ ID NO: 11)

C24 hMSH6 (human cDNA) ACCESSION U28946 (SEQ ID NO: 22)

At page 46, substitute the following three headings:

C25 hPMSR2 (human cDNA) ACCESSION U38964 (SEQ ID NO: 12)

C26 hPMSR2 (human protein) ACCESSION U38964 (SEQ ID NO: 23)

C27 HPMSR3 (human cDNA) ACCESSION U38979 (SEQ ID NO: 13)

At page 47, substitute the following three headings:

C28 hPMSR3 (human protein) ACCESSION U38979 (SEQ ID NO: 24)

C29 hPMSL9 (human cDNA) ACCESSION NM_005395 (SEQ ID NO: 14)

C30 hPMSL9 (human protein) ACCESSION NM_005395 (SEQ ID NO: 25)